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     (FILE 'HOME' ENTERED AT 11:54:14 ON 10 OCT 2001)

     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 11:54:27 ON
     10 OCT 2001
               SEA (SIALIC ACID OR N-ACETYL NEURAMINIC ACID OR NANA)
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                80    FILE ADISALERTS
                 9    FILE ADISINSIGHT
                 2*   FILE ADISNEWS
               781    FILE AGRICOLA
               172    FILE ANABSTR
               442    FILE AQUASCI
               217    FILE BIOBUSINESS
                12    FILE BIOCOMMERCE
             16084    FILE BIOSIS
               362    FILE BIOTECHABS
               362    FILE BIOTECHDS
              3605    FILE BIOTECHNO
              4202    FILE CABA
              3123    FILE CANCERLIT
             17978    FILE CAPLUS
                81    FILE CEABA-VTB
                23    FILE CEN
                26    FILE CIN
               261    FILE CONFSCI
                19    FILE CROPB
                32    FILE CROPU
               584    FILE DDFB
               476    FILE DDFU
               771    FILE DGENE
               584    FILE DRUGB
                 4    FILE DRUGLAUNCH
                 1    FILE DRUGMONOG2
                 3    FILE DRUGNL
               663    FILE DRUGU
                 7    FILE DRUGUPDATES
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              2358    FILE ESBIOBASE
                 2    FILE FOMAD
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               276    FILE FSTA
               462    FILE GENBANK
                21    FILE HEALSAFE
               362    FILE IFIPAT
              1151    FILE JICST-EPLUS
                12    FILE KOSMET
              2937    FILE LIFESCI
                 2    FILE MEDICONF
             14122    FILE MEDLINE
               104    FILE NIOSHTIC
               121    FILE NTIS
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              133   FILE OCEAN
             3057   FILE PASCAL
               12   FILE PHAR
               23   FILE PHIN
              755   FILE PROMT
             7993   FILE SCISEARCH
                2   FILE SYNTHLINE
             1399   FILE TOXLINE
             2346   FILE TOXLIT
             2344   FILE USPATFULL
              835   FILE WPIDS
              835   FILE WPINDEX
L1               QUE (SIALIC ACID OR N-ACETYL NEURAMINIC ACID OR NANA)
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     FILE 'CAPLUS, BIOSIS, MEDLINE, EMBASE, SCISEARCH' ENTERED AT 11:58:39 ON
     10 OCT 2001
L2          5867 S L1 (S) (BIOSYNTHE? OR SYNTHE?)
L3           479 S L2 (P) (ALDOLASE OR SYNTHETASE OR EPIMERASE)
L4             0 S L3 (P) SYNECHOCYSTIS
L5            44 S L3 (P) (E.COLI OR CORYNEBACTERIUM)
L6            19 DUP REM L5 (25 DUPLICATES REMOVED)

=> d l6 ibib ab 1-19


L6   ANSWER 1 OF 19  BIOSIS  COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER:    2001:290213  BIOSIS
DOCUMENT NUMBER:     PREV200100290213
TITLE:               Oligosaccharide enzyme substrates and inhibitors: methods
                     and compositions.
AUTHOR(S):           Wong, Chi-Huey (1); Ichikawa, Yoshitaka; Shen, Gwo-Jenn
CORPORATE SOURCE:    (1) San Diego, CA USA
                     ASSIGNEE: The Scripps Research Institute
PATENT INFORMATION:  US 6168934 January 02, 2001
SOURCE:              Official Gazette of the United States Patent and Trademark
                     Office Patents, (Jan. 2, 2001) Vol. 1242, No. 1, pp. No
                     Pagination. e-file.
                     ISSN: 0098-1133.
DOCUMENT TYPE:       Patent
LANGUAGE:            English
AB   Oligosaccharide compounds that are substrates and inhibitors of
     glycosyltransferase and glycosidase enzymes and compositions containing
     such compounds are disclosed. A method of glycosylation is also
disclosed.
     An E. coli transformed with phagemid CMPSIL-1, which
     phagemid comprises a gene for a modified CMP-sialic acid
     synthetase enzyme, which transformed E. coli
     has the ATCC accession No. 68531 is also provided.

L6   ANSWER 2 OF 19  CAPLUS  COPYRIGHT 2001 ACS        DUPLICATE 1
ACCESSION NUMBER:        2001:527115  CAPLUS
TITLE:                   Redirection of sialic acid metabolism in genetically
                         engineered Escherichia coli
AUTHOR(S):               Ringenberg, Michael; Lichtensteiger, Carol; Vimr,
Eric
CORPORATE SOURCE:        Department of Pathobiology, College of Veterinary
                         Medicine, University of Illinois at Urbana-Champaign,
                         Urbana, IL, 61802, USA
SOURCE:                  Glycobiology (2001), 11(7), 533-539
                         CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER:               Oxford University Press
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Most microorganisms do not produce sialic acid
     (sialate), and those that do appear to use a biosynthetic
```

mechanism distinct from mammals. Genetic hybrids of nonpathogenic, sialate-neg. lab. [redacted]cherichia coli K-12 strains des[redacted]ned for the de novo synthesis of the p[redacted]ysialic acid capsule from **E. co[redacted]** K1 proved useful in elucidating the genetics and biochem. of capsule biosynthesis. In this article we propose a dynamic model of sialometabolism to investigate the effects of biosynthetic neu (N-acetylneuraminic acid) and catabolic nan (N-acylneuraminate) mutations on the flux of intermediates through the sialate synthetic pathway. Intracellular sialate concns. were detd. by high pH anion exchange chromatog. with pulsed amperometric detection. The results indicated that a strain carrying a null defect in the gene encoding polysialytransferase (neuS) accumulated > 50 times more CMP-sialic acid than the wild type when strains were grown in a minimal medium supplemented with glucose and casamino acids. Metabolic accumulation of CMP-sialic acid depended on a functional sialic acid synthase (neuB), as shown by the inability of a strain lacking this enzyme to accumulate a detectable endogenous sialate pool. The neuB mutant concd. trace sialate from the medium, indicating its potential value for quant. anal. of free sialic acids in complex biol. samples. The function of the sialate **aldolase** (encoded by **nanA**) in limiting intermediate flux through the **synthetic** pathway was detd. by analyzing free sialate accumulation in neuA (CMP-**sialic acid synthetase**) **nanA** double mutants. The combined results demonstrate how **E. coli** avoids a futile cycle in which biosynthetic sialate induces the system for its own degrdn. and indicate the feasibility of generating sialooligosaccharide precursors through targeted manipulation of sialate metab.

REFERENCE COUNT: 28

REFERENCE(S):
(1) Barrallo, S; FEBS Lett 1999, V445, P325 CAPLUS
(2) Ferrero, M; Biochem J 1996, V317, P157 CAPLUS
(3) Gilbert, M; Nature Biotechnol 1998, V16, P769 CAPLUS
(4) Hoffman, B; Protein Exp Purif 1995, V6, P646 CAPLUS
(5) Hoyer, L; Mol Microbiol 1992, V6, P873 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:628244 CAPLUS
DOCUMENT NUMBER: 133:218534
TITLE: Human glycosylation enzymes and cDNAs and their use in drug screening, diagnosis, and therapy
INVENTOR(S): Coleman, Timothy A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052136	A2	20000908	WO 2000-US5325	20000301
WO 2000052136	A3	20001228		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000033884 A5 20000921 AU 2000-33884 20000301

PRIORITY APPLN. INFO.: US 1999-122409 P 19990302
WO 2000-US5325 W 20000301

AB The present invention relates to novel human glycosylation enzymes and isolated nucleic acids contg. the coding regions of the genes encoding such enzymes. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human glycosylation enzymes. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human glycosylation enzyme polypeptides. Thus, a human cDNA encoding a protein with significant sequence homol. to mouse CMP N-acetylneuraminic acid **synthetase** was cloned and sequenced. This gene was expressed primarily in colon tissue. Another human cDNA encoded a protein with significant sequence homol. to C. jejuni cytidine **sialic acid synthetase**. A third human cDNA encoding a protein with significant sequence homol. to **E. coli** N-acetylneuraminic acid **aldolase** was cloned and sequenced. This gene was expressed primarily in immune cells and tissues such as primary dendritic cells, monocytes, and bone marrow.

L6 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:351686 CAPLUS
DOCUMENT NUMBER: 133:3768
TITLE: Low cost enzymatic biosynthesis of oligosaccharides
INVENTOR(S): Defrees, Shawn; Johnson, Karl
PATENT ASSIGNEE(S): Neose Technologies, Inc., USA
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029603	A2	20000525	WO 1999-US27599	19991118
WO 2000029603	A3	20001116		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000018261	A5	20000605	AU 2000-18261	19991118
EP 1131415	A2	20010912	EP 1999-961744	19991118

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: US 1998-109031 P 19981118
US 1998-109096 P 19981119
WO 1999-US27599 W 19991118

AB This invention provides recombinant cells, reaction mixts., and methods for the enzymic synthesis of saccharides. The recombinant cells contain a heterologous gene that encodes a glycosyltransferase which catalyzes at least one step of the enzymic synthesis, as well a system for generating a nucleotide sugar that can serve as a substrate for the glycosyltransferase. The nucleotide sugar may be supplied or synthesized by an enzymic pathway comprising a sugar nucleotide regeneration cycle.

The reaction mixt. may contain a second cell type producing a nucleotide as a substrate for the sugar nucleotide regeneration cycle, preferably by a nucleotide synthase gene. Use of fusion proteins of glycosyltransferase and nucleotide sugar synthase combined with the use of an enzyme for substrate sugar synthesis is described. Chem. or enzymic sulfation may be used for the synthesis of sulfated sugars. The recombinant cells, reaction mixts., and methods are useful for efficiently synthesizing a large variety of saccharides, including polysaccharides, oligosaccharides, glycoproteins and glycolipids, using relatively low-cost starting materials. **Synthesis** of 3'-sialyllactose using **E. coli** expressing a CMP-**sialic acid synthetase**/.alpha.2,3-sialyltransferase fusion protein is described. Optional use of bakers yeast to produce CTP used in the sialic acid cycle and substrate for CMP-sialic acid synthase is also described. **Synthesis** of 3'-sialyllactose using **E. coli** expressing a CMP-**sialic acid synthetase** /.alpha.2,3-sialyltransferase fusion protein, GlcNAc 2'-**epimerase** , and **sialic acid aldolase** to **synthesize** CMP-**sialic acid** from GlcNAc is also described. Variations of the method using **Corynebacterium** expressing a CMP-**sialic acid synthetase** /.alpha.2,3-sialyltransferase fusion protein and CTP-**synthetase** to produce the nucleotide, nucleotide sugar, and catalyzing sugar transfer to the acceptor saccharide is described. Finally, synthesis of trisaccharide Gal.alpha.1,3Gal.beta.1,4GlcNAc using **Corynebacterium** expressing UDP-glucose pyrophosphorylase, UDP-glucose-4'-**epimerase**, .beta.1,4-galactosyltransferase, and .alpha.1,3-galactosyltransferase is described.

L6 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2

ACCESSION NUMBER: 2000:202302 CAPLUS
DOCUMENT NUMBER: 133:115759
TITLE: Multiple N-acetyl neuraminic acid synthetase (neuB) genes in Campylobacter jejuni: identification and characterization of the gene involved in sialylation of lipo-oligosaccharide
AUTHOR(S): Linton, Dennis; Karlyshev, Andrey V.; Hitchen, Paul G.; Morris, Howard R.; Dell, Anne; Gregson, Norman A.; Wren, Brendan W.
CORPORATE SOURCE: Department of Neurology, United Medical and Dental School, Guy's Hospital, London, SE1 9RT, UK
SOURCE: Mol. Microbiol. (2000), 35(5), 1120-1134
CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB N-acetylneuraminic acid (NANA) is a common constituent of Campylobacter jejuni lipo-oligosaccharide (LOS). Such structures often mimic human gangliosides and are thought to be involved in the triggering of Guillain-Barre syndrome (GBS) and Miller-Fisher syndrome (MFS) following C. jejuni infection. Anal. of the C. jejuni NCTC 11168 genome sequence identified 3 putative **NANA synthetase** genes termed neuB1, neuB2 and neuB3. The **NANA synthetase** activity of all three C. jejuni neuB gene products was confirmed by complementation expts. in an Escherichia coli neuB-deficient strain. Isogenic mutants were created in all three neuB genes, and for one such mutant (neuB1) LOS was shown to have increased mobility. C. jejuni NCTC 11168 wild-type LOS bound cholera toxin, indicating the presence of NANA in a LOS structure mimicking the ganglioside GM1. This property was lost in the neuB1

mutant. Gas chromatog.-mass spectrometry and fast atom bombardment-mass spectrometry anal. of LOS from wild-type and the neuB1 mutant strain demonstrated the lack of NANA in the latter. Expression of the neuB1 gene in **E. coli** confirmed that NeuB1 was capable of in vitro **NANA biosynthesis** through condensation of N-acetyl-D-mannosamine and phosphoenolpyruvate. Southern anal. demonstrated that the neuB1 gene was confined to strains of C. jejuni with LOS contg. a single NANA residue. Mutagenesis of neuB2 and neuB3 did not affect LOS, but neuB3 mutants were aflagellate and non-motile. No phenotype was evident for neuB2 mutants in strain NCTC 11168, but for strain G1 the flagellin protein from the neuB2 mutant showed an apparent redn. in mol. size relative to the wild type. Thus, the neuB genes of C. jejuni appear to be involved in the biosynthesis of at least 2 distinct surface structures: LOS and flagella.

REFERENCE COUNT: 55

REFERENCE(S):
(1) Altschul, S; Nucleic Acids Res 1997, V25, P3389 CAPLUS
(2) Annunziato, P; J Bacteriol 1995, V177, P312 CAPLUS
(3) Aspinall, G; Biochemistry 1994, V33, P250 CAPLUS
(4) Aspinall, G; Eur J Biochem 1993, V213, P1017 CAPLUS
(5) Aspinall, G; Eur J Biochem 1993, V213, P1029 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3

ACCESSION NUMBER: 1999:714154 CAPLUS
DOCUMENT NUMBER: 132:1660
TITLE: Identification of Arg-12 in the active site of Escherichia coli K1 CMP-sialic acid synthetase
AUTHOR(S): Stoughton, Daniel M.; Zapata, Gerardo; Picone, Robert; Vann, Willie F.
CORPORATE SOURCE: Laboratory of Bacterial Toxins, Division of Bacterial Products, OVRR, CBER, FDA, Bethesda, MD, 20892, USA
SOURCE: Biochem. J. (1999), 343(2), 397-402
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Escherichia coli K1 CMP-sialate **synthetase** (I) catalyzes the **synthesis** of CMP-**sialic acid** from CTP and **sialic acid**. The active site of 418-amino-acid **E. coli** I was localized to its N-terminal half. The bacterial I enzymes have a conserved motif, IAIIPARXXSKGLXXKN, at their N-termini. Several basic residues were identified at or near the active site of the **E. coli** enzyme by chem. modification and site-directed mutagenesis. Only 1 of the Lys residues in the N-terminal motif, Lys-21, appeared to be essential for activity. Mutation of Lys-21 in the N-terminal motif resulted in an inactive enzyme. Furthermore, Arg-12 of the N-terminal motif appeared to be an active site residue, based on the following evidence. Substituting Arg-12 with Gly or Ala resulted in inactive enzymes, indicating that this residue is required for enzymic activity. The R12K mutant was partially active, demonstrating that a pos. charge is required at this site. Steady-state kinetic anal. revealed changes in kcat, Km, and Ks values for CTP, which implicated Arg-12 in catalysis and substrate binding.

REFERENCE COUNT: 25

REFERENCE(S):
(1) Ambrose, M; Biochemistry 1992, V31, P775 CAPLUS
(2) Caligiuri, M; J Biol Chem 1991, V266, P8328 CAPLUS
(4) Ganguli, S; J Bacteriol 1994, V176, P4583 CAPLUS

(5) Guerry, P; Mol Microbiol 1996, V19, P369 CAPLUS
(8) Jelakovic, S; FEBS Lett 19[redacted] V391, P157 CAPLUS
ALL CITATIONS AVAILABLE IN THE [redacted] FORMAT

L6 ANSWER 7 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:415986 BIOSIS
DOCUMENT NUMBER: PREV199800415986
TITLE: Characterization of the CTP binding sites in the **E**
. **coli** K1 CMP-**sialic acid**
synthetase.
AUTHOR(S): Stoughton, D. M.; Vann, W. F.
CORPORATE SOURCE: Food Drug Administration, Bethesda, MD USA
SOURCE: Abstracts of the General Meeting of the American Society
for Microbiology, (1998) Vol. 98, pp. 125.
Meeting Info.: 98th General Meeting of the American
Society
for Microbiology Atlanta, Georgia, USA May 17-21, 1998
American Society for Microbiology
. ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L6 ANSWER 8 OF 19 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:408767 SCISEARCH
THE GENUINE ARTICLE: ZK302
TITLE: Arginine-12 is essential for CTP binding and catalysis of
the **E-coli** K1 CMP-**sialic**
acid synthetase
AUTHOR: Stoughton D M (Reprint); Zapata G; Vann W F
CORPORATE SOURCE: FDA, CBER, BETHESDA, MD
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (31 JUL 1997) Vol. 11, No. 9, Supp. [S],
pp. 3303-3303.
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE
PIKE, BETHESDA, MD 20814-3998.
ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L6 ANSWER 9 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1997:422568 BIOSIS
DOCUMENT NUMBER: PREV199799721771
TITLE: Arginine-12 is essential for CTP binding and catalysis of
the **E. coli** K1 CMP-**sialic**
acid synthetase.
AUTHOR(S): Stoughton, D. M.; Zapata, G.; Vann, W. F.
CORPORATE SOURCE: CBER, FDA, Bethesda, MD USA
SOURCE: FASEB Journal, (1997) Vol. 11, No. 9, pp. A1421.
Meeting Info.: 17th International Congress of Biochemistry
and Molecular Biology in conjunction with the Annual
Meeting of the American Society for Biochemistry and
Molecular Biology San Francisco, California, USA August
24-29, 1997
ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L6 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4
ACCESSION NUMBER: 1997:508112 CAPLUS
DOCUMENT NUMBER: 127:216834
TITLE: Purification and characterization of the Escherichia
coli K1 neuB gene product N-acetylneuraminic acid
synthetase
AUTHOR(S): Vann, Willie F.; Tavarez, Jose J.; Crowley, Jane;

Vimr, Eric; Silver, Richard P.
CORPORATE SOURCE: Laboratory of Bacterial Polysaccharides, Center for Biologics Research and Review, Bethesda, MD, 20892, USA
SOURCE: Glycobiology (1997), 7(5), 697-701
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli K1 produces a capsular polysaccharide of .alpha.(2-8)-poly-N-acetylneuraminic acid. This polysaccharide is an essential virulence factor of these neuropathogenic bacteria. The genes necessary for the synthesis of neuNAc were localized to a plasmid contg. the neuBAC genes of the K1 gene cluster. Cells harboring the neuB+ allele in an **aldolase** (nanA-) neg. background produce neuNAc in vivo. Enzymic synthesis of neuNAc could be demonstrated in exts. of cells harboring an expression plasmid (pNEUB) contg. the neuB gene alone. NeuNAc **synthetase** was purified to homogeneity from exts. of cells harboring pNEUB. The mol. wt. of the purified enzyme is 40 kDa, similar to that predicted by the nucleotide sequence of the neuB gene. The N-terminal sequence of the purified protein matches that predicted by the nucleotide sequence of the neuB gene. NeuNAc **synthetase** catalyzes the formation of neuNAc as indicated by its coupling to the CMP-NeuNAc **synthetase** reaction. The enzyme condenses ManNAc and PEP with the release of phosphate. The **E.coli** neuNAc **synthetase** is specific for ManNAc and PEP, unlike rat liver enzyme that utilizes N-acetylmannosamine-6-phosphate to form NeuNAc-9-PO4. This represents the first report of a purifn. of a **sialic acid synthetase** from either a eukaryotic or prokaryotic source to homogeneity. These expts. clearly demonstrate an **aldolase**-independent **sialic acid synthetase** activity in **E. coli** K1.

L6 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:486007 CAPLUS
TITLE: Chemo-enzymic synthesis and structural analysis of polysialic acid.
AUTHOR(S): McGowen, M. M.; Jennings, H. J.; Vann, W. F.
CORPORATE SOURCE: CBER, FDA, Bethesda, MD, 20892, USA
SOURCE: Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7-11 (1997), CARB-051. American Chemical Society: Washington, D. C.
CODEN: 64RNAO
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Poly-.alpha.(2,8)-N-acetyl neuraminic acid (polysialic acid, PSA) has been implicated in the pathogenic mechanism of Escherichia coli K1 urinary tract infection and neonatal meningitis. The three dimensional structure of PSA is rationalized as random coil with localized helixes of n = 9-11. The carboxylate group is essential to stabilizing the active conformation of PSA. In this study we are investigating the role that the hydroxyl moiety at C9 plays. Our approach was to **synthesize** **sialic acid** analogs modified at C9 by chem. methods and incorporate analogs into polymer by using enzymes. 9-Azidosialic acid was charged with CMP using recombinant CMP-NeuNAc **synthetase**. Mutant **E. coli** K1, EV241, which can neither **synthesize** nor degrade **sialic acid** was the source of sialyltransferase. Incubation of the activated azido-monomer with total membrane prepns. of EV241 enzymically synthesized modified .alpha.(2,8)-PSA de novo. The 9-modified PSA was isolated and purified by gel filtration and anion exchange chromatog. The homopolysaccharide was then confirmed by 13C NMR spectroscopy and Dionex integrated amperometry

HPLC anal. The data presented may assist in the further understanding of the active confor of PSA involved in pathogenesi or immunol. response.

L6 ANSWER 12 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5
ACCESSION NUMBER: 1996:154855 BIOSIS
DOCUMENT NUMBER: PREV199698726990
TITLE: Characterization of cpsF and its product CMP-N-acetylneuraminic acid synthetase, a group B streptococcal enzyme that can function in K1 capsular polysaccharide biosynthesis in Escherichia coli.
AUTHOR(S): Haft, Rachel F.; Wessels, Michael R. (1); Mebane, Mary Fisk; Conaty, Neil; Rubens, Craig E.
CORPORATE SOURCE: (1) Channing Lab., Brigham Women's Hosp., Div. Infectious Diseases, Beth Israel Hosp., Harvard Med. Sch., 180 Longwood Avenue, Boston, MA 02115 USA
SOURCE: Molecular Microbiology, (1996) Vol. 19, No. 3, pp. 555-563.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Group B Streptococcus (GBS) is the foremost cause of neonatal sepals and meningitis in the United States. A major virulence factor for GBS is its capsular polysaccharide, a high molecular weight polymer of branched oligosaccharide subunits. N-acetylneuraminic acid (Neu5Ac or **sialic acid**), at the end of the polysaccharide side chains, is critical to the virulence function of the capsular polysaccharide. Neu5Ac must be activated by CMP-Neu5Ac **synthetase** before it is incorporated into the polymer. We showed previously that a transposon mutant of a serotype III GBS strain which had no detectable capsular Neu5Ac was deficient in CMP-Neu5Ac-**synthetase** activity (Wessels et al., 1992). In this paper, we report the identification and characterization of cpsF, a gene interrupted by transposon insertion in the previously described Neu5Ac-deficient mutant. The predicted amino acid sequence of the cpsF gene product shares 57% similarity and 37% identity with CMP-Neu5Ac **synthetase** encoded by the Escherichia coli K1 gene, neuA. The enzymatic function of the protein encoded by cpsF was established by cloning the gene in **E. coli** under the control of the T7 polymerase/promoter. Lysates of **E. coli** in which the cpsF gene product was expressed, catalysed the condensation of CTP with Neu5Ac to form CMPNeu5Ac. In addition, when a CMP-Neu5Ac **synthetase**-deficient mutant of **E. coli** K1 was transformed with cpsF, K1 antigen expression was restored. We conclude that cpsF encodes CMP-Neu5Ac **synthetase** in type III GBS, and that the GBS enzyme can function in the capsule-**synthesis** of a heterologous bacterial species.

L6 ANSWER 13 OF 19 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 96150083 MEDLINE
DOCUMENT NUMBER: 96150083 PubMed ID: 8579837
TITLE: CMP-N-acetyl neuraminic-acid synthetase from Escherichia coli: fermentative production and application for the preparative synthesis of CMP-neuraminic acid.
AUTHOR: Kittelmann M; Klein T; Kragl U; Wandrey C; Ghisalba O
CORPORATE SOURCE: Ciba-Geigy Ltd, Pharmaceuticals Division, Basel, Switzerland.
SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1995 Dec) 44 (1-2) 59-67.
Journal code: AMC; 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199603

ENTRY DATE: Entered STN: 19960327
Last Updated on STN: 19980206
Entered Medline: 19960321

AB In an optimized sorbitol/yeast extract/mineral salt medium up to 12 U/l CMP-**N-acetyl-neuraminic-acid** (Neu5Ac) **synthetase** was produced by Escherichia coli K-235 in shake-flask culture. A colony mutant of this strain, **E. coli** K-235/CS1, was isolated with improved enzyme formation: in shake flasks with a yield of up to 20.8 U/l and 54 mU/mg protein in the cell extract. With this strain 26500 U CMP-Neu5Ac **synthetase** was produced with a high specific activity (0.128 U/mg) by fed-batch fermentation on 230-l scale. On a 10-l scale the enzyme yield was 191 U/l culture medium. The enzyme was partially purified by precipitation with polyethyleneglycol resulting in a three- to fourfold enrichment and a recovery rate of more than 80%; most of the CTP hydrolysing enzymes were removed. The native **synthetase** was deactivated completely by incubation at 45 degrees C for 10 min, but could be stabilized remarkably by glycerol and different salts. The enzyme was used for the preparative **synthesis** of CMP-Neu5Ac with a conversion yield of 87% based on CTP.

L6 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:146291 CAPLUS
DOCUMENT NUMBER: 118:146291
TITLE: CMP-sialic acids manufacture with microbial cell extracts
INVENTOR(S): Kittelmann, Matthias; Ghisalba, Oreste; Klein, Teresa;
Kragl, Udo; Wandrey, Christian Prof Dr
PATENT ASSIGNEE(S): Ciba-Geigy A.-G., Switz.; Forschungszentrum Juelich GmbH
SOURCE: Eur. Pat. Appl., 25 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 524143	A1	19930120	EP 1992-810522	19920708
EP 524143	B1	19971210		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, PT, SE				
AT 161051	E	19971215	AT 1992-810522	19920708
ES 2110481	T3	19980216	ES 1992-810522	19920708
CA 2073954	AA	19930118	CA 1992-2073954	19920715
AU 9220348	A1	19930121	AU 1992-20348	19920716
AU 664036	B2	19951102		
JP 05276973	A2	19931026	JP 1992-189647	19920716
IL 102527	A1	19960804	IL 1992-102527	19920716
US 5334514	A	19940802	US 1993-152269	19931112

PRIORITY APPLN. INFO.: CH 1991-2119 A 19910717
US 1992-915474 B1 19920716

AB CMP-**sialic acids** are prepd. by incubation of CTP and **sialic acids** with microbial cell exts. contg. cytidine-5'-monophospho-N-acetylneuraminic acid **synthetase** activity. Escherichia coli was cultured and an ext. was prepd. which was used to prep. CMP-Neu5Ac from CTP and N-acetylneuraminic acid (Neu5Ac). Methods for optimizing **E. coli** growth and enzyme yield and for further purifn. of the enzyme were described. An **E. coli** mutant with higher yields of the enzyme was produced.

L6 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 7
ACCESSION NUMBER: 1992:470187 CAPLUS
DOCUMENT NUMBER: 117:70187
TITLE: Overproduction of CMP-sialic acid synthetase for

organic synthesis

AUTHOR(S): Liu, Jennifer Lin Chun; Shen, [redacted] Jenn; Ichikawa, Yoshitaka; Rutan, James F.; Zapata, Gerardo; Vann, Willie F.; Wong, Chi Huey

CORPORATE SOURCE: Dep. Chem., Scripps Res. Inst., La Jolla, CA, 92037, USA

SOURCE: J. Am. Chem. Soc. (1992), 114(10), 3901-10
CODEN: JACSAT; ISSN: 0002-7863

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The gene coding for Escherichia coli CMP-**sialic acid synthetase** (E.C. 2.7.7.43) was cloned and overexpressed in **E. coli** through a primer-directed polymerase chain reaction. Two plasmids were constructed to produce the native enzyme and a modified enzyme fused with a decapeptide at the C-terminus. The decapeptide tag was used for detection of the enzyme prodn. Both enzymes produced from **E. coli** were isolated and purified with an orange A dye resin and FPLC. Contrary to the native enzyme, the modified enzyme is more active at higher pH. Studies on specificity indicate that both enzymes have a high specific activity for C-9 modified NeuAc derivs. at neutral pH. Some C-5 modified (hydroxy-, deoxy-, and deoxyfluoro-) NeuAc derivs. are not acceptable as substrates. The modified enzyme has been used in the synthesis of CMP-NeuAc from ManNAc and CMP and sialyl N-acetyllactosamine (Neu.alpha.2,6Gal.beta.1,4GlcNAc) with in situ generation of NeuAc and regeneration of CMP-NeuAc. The 6-O-acyl derivs. of ManNAc were prepd. via transesterification in anhyd. DMF by using an engineered stable subtilisin variant as a catalyst, and the products were used as substrates in **sialic acid aldolase**-catalyzed **synthesis** of 9-O-acyl-NeuAc derivs.

L6 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 8

ACCESSION NUMBER: 1986:17473 CAPLUS

DOCUMENT NUMBER: 104:17473

TITLE: Regulation of sialic acid metabolism in Escherichia coli: role of N-acylneuraminate pyruvate-lyase

AUTHOR(S): Vimr, Eric R.; Troy, Frederic A.

CORPORATE SOURCE: Sch. Med., Univ. California, Davis, CA, 95616, USA

SOURCE: J. Bacteriol. (1985), 164(2), 854-60
CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In **E. coli**, **synthesis** of **sialic acid** is not regulated by allosteric inhibition mediated by cytidine 5'-monophospho-N-acetylneuraminic acid (CMP-NeuNAc). Evidence for the lack of metabolic control by feedback inhibition was demonstrated by measuring the intracellular levels of **sialic acid** and CMP-NeuNAc in mutants defective in **sialic acid** polymn. and in CMP-NeuNAc **synthesis**. Polymn.-defective mutants could not **synthesize** the poly(**sialic acid**) capsule and accumulated 25-fold more CMP-NeuNAc than the wild type. Mutants unable to activate **sialic acid** because of a defect in CMP-NeuNAc **synthetase** accumulated .apprx.7-fold more **sialic acid** than the wild type. An addnl. 3-fold increase in **sialic acid** levels occurred when a mutation resulting in loss of N-acylneuraminate pyruvate-lyase (**sialic acid aldolase**) was introduced into the CMP-NeuNAc **synthetase**-deficient mutant. The **aldolase** mutation could not be introduced into the polymn.-defective mutant, suggesting that any further increase in the intracellular CMP-NeuNAc concn. was toxic. Thus, sialic acid **aldolase** can regulate the intracellular concn. of sialic acid and, therefore, the concn. of CMP-NeuNAc. Thus, regulation of **aldolase**, mediated by sialic acid induction, is necessary not only for dissimilating sialic acid but also for modulating the level of metabolic intermediates in the sialic acid pathway. In agreement with this conclusion, an increase in the intracellular sialic acid concn. was correlated with an increase in

aldolase activity. Direct evidence for the central role of **aldolase** in regulating the metabolic flux of sialic acid in **E. coli** was that exogenous, radiolabeled sialic acid was specifically incorporated into sialyl polymer in an **aldolase** -neg. strain, but not in the wild type.

L6 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 9
ACCESSION NUMBER: 1986:3295 CAPLUS
DOCUMENT NUMBER: 104:3295
TITLE: Identification of an inducible catabolic system for sialic acids (nan) in Escherichia coli
AUTHOR(S): Vimr, Eric R.; Troy, Frederic A.
CORPORATE SOURCE: Sch. Med., Univ. California, Davis, CA, 95616, USA
SOURCE: J. Bacteriol. (1985), 164(2), 845-53
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli K-12 and K-12 hybrid strains constructed to express a polysialic acid capsule, the K1 antigen, were able to efficiently use sialic acid as a sole C source. This ability was dependent on induction of .gtoreq.2 activities: a sialic acid-specific transport activity, and an **aldolase** activity specific for cleaving sialic acids. Induction over basal levels required sialic acid as the apparent inducer, and induction of both activities was repressed by glucose. Induction also required the intracellular accumulation of **sialic acid**, which could be either added exogenously to the medium or accumulated intracellularly through **biosynthesis**. Exogenous sialic acid appeared to be transported by an active mechanism that did not involve covalent modification of the sugar. Mutations affecting either the transport or degrdn. of sialic acid prevented its use as a C source and have been designated nanT and nanA, resp. These mutations were located by transduction near min 69 on the **E. coli** K-12 genetic map, between argG and glnF. In addn. to being unable to use sialic acid as a C source, **aldolase**-neg. mutants were growth-inhibited by this sugar. Therefore, the intracellularly accumulated sialic acid was toxic in **aldolase**-deficient **E. coli** strains. The dual role of **aldolase** in dissimilating and detoxifying sialic acids is consistent with the apparent multiple controls on expression of this enzyme.

L6 ANSWER 18 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 84202093 EMBASE
DOCUMENT NUMBER: 1984202093
TITLE: Purification and properties of **E. coli** cytidine 5'- monophosphate **n-acetyl-neuraminic acid synthetase**.
AUTHOR: Vann W.F.; Kotsatos M.; Chang K.; et al.
CORPORATE SOURCE: National Center for Drugs and Biologics, FDA, Bethesda, MD 20205, United States
SOURCE: Federation Proceedings, (1984) 43/6 (no. 1644).
CODEN: FEPRA7
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English

L6 ANSWER 19 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1985:245274 BIOSIS
DOCUMENT NUMBER: BA79:25270
TITLE: PURIFICATION AND PROPERTIES OF N ACETYLNEURAMINATE LYASE EC-4.1.3.3 FROM ESCHERICHIA-COLI.
AUTHOR(S): UCHIDA Y; TSUKADA Y; SUGIMORI T
CORPORATE SOURCE: KYOTO RES. LAB., MARUKIN SHOYU CO., LTD., UJI, KYOTO 611.
SOURCE: J BIOCHEM (TOKYO), (1984) 96 (2), 507-522.

CODEN: JOBIAO. ISSN: 0021-924X.
FILE SEGMENT: BA[redacted]LD
LANGUAGE: English

AB N-Acetylneuraminate lyase [N-acetylneuraminic acid **aldolase** EC 4.1.3.3] from **E. coli** was purified by protamine sulfate treatment, fractionation with (NH4)2 SO4, column chromatography on DEAE-Sephacel, gel filtration on Ultrogel AcA 44 and preparative polyacrylamide gel electrophoresis. The purified enzyme preparation was homogenous on analytical polyacrylamide gel electrophoresis, and was free from contaminating enzymes including NADH oxidase and NADH dehydrogenase. The enzyme catalyzed the cleavage of N-acetylneuraminic acid to N-acetylmannosamine and pyruvate in a reversible reaction. Both cleavage and **synthesis** of N-acetylneuraminic acid had the same pH optimum .apprx. 7.7. The enzyme was stable at pH 6.0-9.0, and was thermostable up to 60.degree. C. The thermal stability increased up to 75.degree. C in the presence of pyruvate. No metal ion was required for the enzyme activity, but heavy metal ions such as Ag+ and Hg2+ were potent inhibitors. Oxidizing agents such as N-bromosuccinimide, I, and H2O, and SH-inhibitors such as P-chloromercuribenzoic acid and HgCl2 were also potent inhibitors.

The Km values for N-acetylneuraminic acid and N-glycolylneuraminic acid were 3.6 mM and 4.3 mM, respectively. Pyruvate inhibited the cleavage reaction competitively; K1 was calculated to be 1.0 mM. In the condensation reaction, N-acetylglucosamine, N-acetylgalactosamine, glucosamine and galactosamine could not replace N-acetylmannosamine as substrate, and phosphoenolpyruvate, lactate, .beta.-hydroxypyruvate and other pyruvate derivatives could not replace pyruvate as substrate. The MW of the native enzyme was estimated to be 98,000 by gel filtration methods.

After denaturation in sodium dodecyl sulfate or in 6 m guanidine-HCl, the MW was reduced to 33,000, indicating the existence of 3 identical subunits. The enzyme could be used for the enzymatic determination of **sialic acid**; reaction conditions were devised for determining the bound form of **sialic acid** by coupling neuraminidase from Arthrobacter ureafaciens, lactate dehydrogenase and NADH.

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=> d his

     (FILE 'HOME' ENTERED AT 14:42:25 ON 10 OCT 2001)

     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:42:38 ON
     10 OCT 2001
                SEA (N-ACETYLGLUCOSAMINE 2'-EPIMERASE) OR (UDP-GLCNAC
2'-EPIMER
                ---------
                0*  FILE ADISNEWS
                5   FILE BIOSIS
                1   FILE CANCERLIT
                3   FILE CAPLUS
                1   FILE CONFSCI
                4   FILE EMBASE
                1   FILE LIFESCI
                4   FILE MEDLINE
                3   FILE SCISEARCH
                1   FILE TOXLIT
L1               QUE (N-ACETYLGLUCOSAMINE 2'-EPIMERASE) OR (UDP-GLCNAC
2'-EPIMER
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     FILE 'BIOSIS, EMBASE, MEDLINE, CAPLUS, SCISEARCH' ENTERED AT 14:47:25 ON
     10 OCT 2001
L2            19 S L1
L3             6 DUP REM L2 (13 DUPLICATES REMOVED)
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=> d l3 ibib ab 1-6

L3 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1999:311054 BIOSIS
DOCUMENT NUMBER: PREV199900311054
TITLE: Mutations in the human UDP-N-acetylglucosamine 2-epimerase gene define the disease sialuria and the allosteric site of the enzyme.
AUTHOR(S): Seppala, Raili; Lehto, Veli-Pekka; Gahl, William A. (1)
CORPORATE SOURCE: (1) NICHD, NIH, 10 Center Drive, Building 10, Room 9S-241, Bethesda, MD, 20892-1830 USA
SOURCE: American Journal of Human Genetics, (June, 1999) Vol. 64, No. 6, pp. 1563-1569.
ISSN: 0002-9297.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Sialuria is a rare inborn error of metabolism characterized by cytoplasmic accumulation and increased urinary excretion of free N-acetylneuraminic acid (NeuAc, sialic acid). Overproduction of NeuAc is believed to result from loss of feedback inhibition of uridinediphosphate-**N-acetylglucosamine 2-epimerase** (**UDP-GlcNAc 2-epimerase**) by cytidine monophosphate-N-acetylneuraminic acid (CMP-Neu5Ac). We report the cloning and characterization of human UDP-GlcNAc 2-epimerase cDNA, with mutation analysis of three patients with sialuria. Their heterozygote mutations, R266W, R266Q, and R263L, indicate that the allosteric site of the epimerase resides in the region of codons 263-266. The heterozygous nature of the mutant allele in all three patients reveals a dominant mechanism of inheritance for sialuria.

L3 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2
ACCESSION NUMBER: 1999:397525 BIOSIS
DOCUMENT NUMBER: PREV199900397525
TITLE: UDP-GlcNAc 2-epimerase: A regulator of cell surface sialylation.
AUTHOR(S): Keppler, Oliver T.; Hinderlich, Stephan; Langner, Josmar; Schwartz-Albiez, Reinhard; Reutter, Werner; Pawlita, Michael (1)
CORPORATE SOURCE: (1) Applied Tumor Virology Program, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120, Heidelberg Germany
SOURCE: Science (Washington D C), (May 21, 1999) Vol. 284, No. 5418, pp. 1372-1376.
ISSN: 0036-8075.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Modification of cell surface molecules with sialic acid is crucial for their function in many biological processes, including cell adhesion and signal transduction. Uridine diphosphate-**N-acetylglucosamine 2-epimerase** (**UDP-GlcNAc 2-epimerase**) is an enzyme that catalyzes an early, rate-limiting step in the sialic acid biosynthetic pathway. UDP-GlcNAc 2-epimerase was found to be a major determinant of

cell surface sialylation in human hematopoietic cell lines and a critical regulator of the [redacted]ction of specific cell surface [redacted]hesion molecules.

L3 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 3
ACCESSION NUMBER: 1997:486001 BIOSIS
DOCUMENT NUMBER: PREV199799785204
TITLE: A bifunctional enzyme catalyzes the first two steps in N-acetylneuraminic acid biosynthesis of rat liver: Molecular cloning and functional expression of UDP-N-acetyl-glucosamine 2-epimerase/N-acetylmannosamine kinase.
AUTHOR(S): Staesche, Roger; Hinderlich, Stephan; Weise, Christoph; Effertz, Karin; Lucka, Lothar; Moormann, Petra; Reutter, Werner (1)
CORPORATE SOURCE: (1) Inst. Molekularbiol. Biochemie, Freie Univ. Berlin, Arnimallee 22, D-14195 Berlin-Dahlem Germany
SOURCE: Journal of Biological Chemistry, (1997) Vol. 272, No. 39, pp. 24319-24324.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB N-Acetylneuraminic acid (Neu5Ac) is the precursor of sialic acids, a group of important molecules in biological recognition systems. Biosynthesis of Neu5Ac is initiated and regulated by its key enzyme, UDP-**N-acetylglucosamine 2-epimerase** (**UDP-GlcNAc 2-epimerase**, EC 5.1.3.14)/N-acetylmannosamine kinase (ManNAc kinase, EC 2.7.1.60) in rat liver (Hinderlich, S., Stasche, R., Zeitler, R., and Reutter, W. (1997) J. Biol. Chem. 272, 24313-24318). In the present paper we report the isolation and characterization of a cDNA clone encoding this bifunctional enzyme. An open reading frame of 2166 base pairs encodes 722 amino acids with a predicted molecular mass of 79 kDa. The deduced amino acid sequence contains exact matches of the sequences of five peptides derived from tryptic cleavage of the enzyme. The recombinant bifunctional enzyme was expressed in COS7 cells, where it displayed both epimerase and kinase activity. Distribution of UDP-GlcNAc 2-epimerase/ManNAc kinase in the cytosol of several rat tissues was investigated by determining both specific enzyme activities. Secreting organs (liver, salivary glands, and intestinal mucosa) showed high specific activities of UDP-GlcNAc 2-epimerase/ManNAc kinase, whereas significant levels of these activities were absent from other organs (lung, kidney, spleen, brain, heart, skeletal muscle, and testis). Northern blot analysis revealed no UDP-GlcNAc 2-epimerase/ManNAc kinase mRNA in the non-secreting tissues.

L3 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1997:486000 BIOSIS
DOCUMENT NUMBER: PREV199799785203
TITLE: A bifunctional enzyme catalyzes the first two steps in N-acetylneuraminic acid biosynthesis of rat liver: Purification and characterization of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase.
AUTHOR(S): Hinderlich, Stephan; Staesche, Roger; Zeitler, Reinhard; Reutter, Werner (1)
CORPORATE SOURCE: (1) Inst. Molekularbiol. Biochemie, Freie Univ. Berlin, Arnimallee 22, D-14195 Berlin-Dahlem Germany
SOURCE: Journal of Biological Chemistry, (1997) Vol. 272, No. 39, pp. 24313-24318.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Biosynthesis of N-acetylneuraminic acid (Neu5Ac), a prominent component of glycoconjugates, is initiated by the action of UDP-**N-acetylglucosamine 2-epimerase** (**UDP-**

GlcNAc 2-epimerase, EC 5.1.3.14) and N-acetylmannosamine kinase (ManNAc kinase, EC 2.7.1.60). We demonstrate for the first time that the two activities are part of one bifunctional enzyme in rat liver. The enzyme was purified to homogeneity from rat liver cytosol using salmine sulfate precipitation and chromatography on phenyl-Sepharose, ATP-agarose, and Mono Q. The purification resulted in one polypeptide with an apparent molecular mass of 75 kDa. Immunoprecipitation with a polyclonal antibody against the polypeptide reduced both enzyme activities in equal amounts. Gel filtration analysis of purified UDP-GlcNAc 2-epimerase/ManNAc kinase showed that the polypeptide self-associates as a dimer and as a hexamer with apparent molecular masses of 150 and 450 kDa, respectively. The hexamer was fully active for both enzyme activities, whereas the dimer catalyzed only the phosphorylation of N-acetylmannosamine (ManNAc). Incubation of the dimer with UDP-N-acetylglucosamine led to reassembly of the fully active hexamer; maximal quantities of the hexamer were produced after incubation for 3 h. Kinetic analysis of purified hexameric and dimeric enzyme revealed significantly lower Michaelis constants (93 +- 3 to 121 +- 15 mu-M for ManNAc and 1.18 +- 0.13 to 1.67 +- 0.20 mM for ATP) and higher cooperativity (Hill coefficients of 1.42 +- 0.16 to 1.17 +- 0.06 for ManNAc and 1.30 +- 0.09 to 1.05 +- 0.14 for ATP) for the hexamer for both substrates of ManNAc kinase. The Michaelis constant of UDP-GlcNAc 2-epimerase for its substrate was 11 +- 2 mu-M. The Hill coefficient of 0.45 +- 0.07 represents strongly negative cooperativity in substrate binding. UDP-GlcNAc 2-epimerase was feedback inhibited by CMP-Neu5Ac. Complete inhibition was achieved with 60 mu-M CMP-Neu5Ac, and highly positive cooperativity (Hill coefficient of 4.1) was found for inhibitor binding.

L3 ANSWER 5 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:76055 SCISEARCH
THE GENUINE ARTICLE: YQ995
TITLE: Molecular cloning of the rate-limiting enzyme in sialic acid synthesis, uridinediphosphate-**N-acetylglucosamine-2-epimerase** (**UDP-GlcNAc-2-epimerase**): Implications for sialuria.
AUTHOR: Seppala R (Reprint); Lehto V P; Gahl W A
CORPORATE SOURCE: NICHHD, SECT HUMAN BIOCHEM GENET, HERITABLE DISORDERS BRANCH, NIH, BETHESDA, MD; UNIV OULU, DEPT PATHOL, OULU, FINLAND
COUNTRY OF AUTHOR: USA; FINLAND
SOURCE: AMERICAN JOURNAL OF HUMAN GENETICS, (OCT 1997) Vol. 61, No. 4, Supp. [S], pp. 1519-1519.
Publisher: UNIV CHICAGO PRESS, 5720 S WOODLAWN AVE, CHICAGO, IL 60637.
ISSN: 0002-9297.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 0

L3 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:111440 BIOSIS
DOCUMENT NUMBER: PREV199800111440
TITLE: Molecular cloning of the rate-limiting enzyme in sialic acid synthesis, uridinediphosphate-**N-acetylglucosamine-2-epimerase** (**UDP-GlcNAc-2-epimerase**): Implications for sialuria.
AUTHOR(S): Seppala, R. (1); Lehto, V. P.; Gahl, W. A.
CORPORATE SOURCE: (1) Section Human Biochem. Genetics, Heritable Disorders Branch, NICHD, National Inst. Health, Bethesda, MD USA
SOURCE: American Journal of Human Genetics, (Oct., 1997) Vol. 61, No. 4 SUPPL., pp. A261.

Meeting Info.: 47th Annual Meeting of the American Society of [redacted]man Genetics Baltimore, Maryla USA October 28 [redacted]ovember 1, 1997
ISSN: 0002-9297.
DOCUMENT TYPE: Conference
LANGUAGE: English